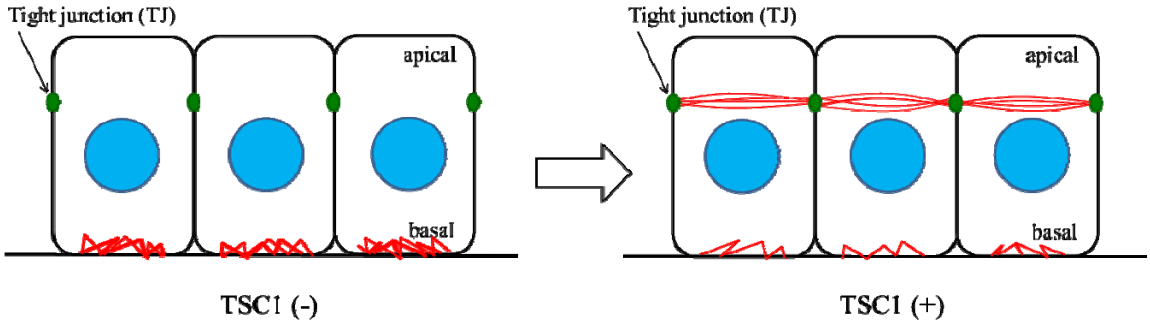
論文の内容の要旨

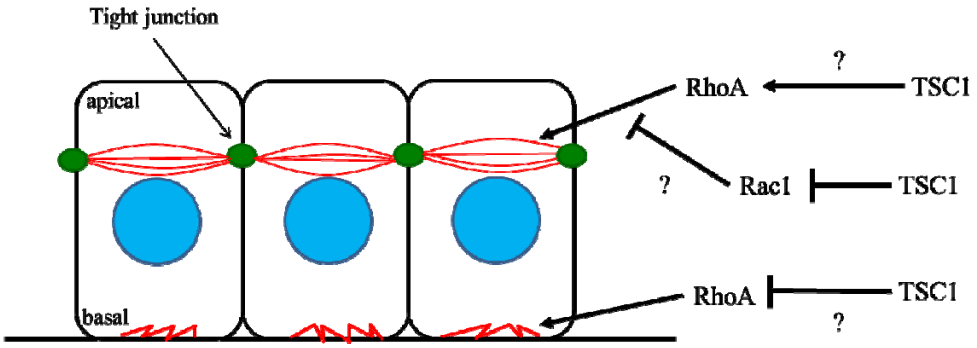
論文題目　Product of TSC1, causative gene of tuberous sclerosis, regulates intracellular distribution of actin cytoskeleton according to cell polarity.
（結節性硬化症の原因遺伝子 TSC1 の生成物は細胞極性に応じてアクチン細胞骨格の細胞内分布を制御している。）

大澤　麻記

Tuberous sclerosis complex (TSC) is an autosomal dominant neurocutaneous syndrome caused by mutations in either of the two tumor suppressor genes *TSC1* or *TSC2*, which encode TSC1 (hamartin) and TSC2 (tuberin), respectively. TSC is clinically characterized by seizures, mental retardation, behavioral disturbances, and hamartomatous lesions in many organs. TSC1 and TSC2 form a complex that negatively regulates the mammalian target of rapamycin complex 1 (mTORC1) activity by inhibiting Rheb. The active form of Rheb enhances phosphorylation of mTORC1 substrates S6K and 4E-BP1, and promotes cell growth and proliferation. In upstream of TSC1/2, Akt is one of the inhibitor of TSC1/2. The different evidences for separable functions of TSC1 and TSC2 have been provided. However, very little is known about the function of TSC1 compared with TSC2. The Rho family of small GTPases (Rho, Rac and Cdc42) have effects on the actin cytoskeleton, and play a role in cell polarity, migration and proliferation. Recent studies suggest that TSC1 and TSC2 regulate the activities of Rho family small GTPases, Rac1 and Rho, and the ensuing actin cytoskeletal organization at focal adhesion. However, how TSC1 contributes to the establishment of cell polarity has not been well known. Here, I analyzed TSC1 function focusing on small GTPase and actin fiber remodeling, using originally established mice renal tumor cell line. First, I established stable TSC1-expressing cell lines from a *Tsc1*-deficient mouse renal tumor cell line. mTORC1 signaling pathway activated by

Tsc1-deficiency cells was suppressed by restoration of TSC1. I also observed the increase in p-Akt levels by TSC1 expression, indicating that a negative feedback loop was enhanced during renal tumorigenesis in *Tsc1* knockout mice. TSC1 inhibited cell proliferation, but not cell growth in this study. Cell migration was suppressed when TSC1 was expressed. In addition, Rac1 activity, as well as the formation of lamellipodia and filopodia, was decreased. These results indicate that cell migration was inhibited by TSC1, which likely result from the reduction of filopodia and focal adhesion. To my knowledge, this is the first report of TSC1 function on cell migration in mammalian epithelial cells. In the confluent monolayer, apical actin fibers which aligned and traversing the cell body from one side to the other appeared in the apical region of TSC1-expressing cells. This apical actin network seemed to connect with cortical actin at the level of tight junction. A report demonstrated that radially organized actin fibers appeared under the condition of inhibition of cell-cell junction formation. In contrast, cell-cell junction in TSC1-expressing cell was not remarkably changed from that in TSC1-deficient cells in my result. I speculate that the formation of apical actin network is not directly related to the assembly and disassembly of cell-cell junctional complexes. From the observation that fibers were arranged as if they were intercellularly connected, functions of apical actin network are suggested to be coordinated between different cells. Thus, apical actin network found in TSC1-expressing cells might be a structure for the regulation of cell morphology or permeability in renal epithelial cells. This apical actin network was markedly decreased by ROCK inhibitor treatment. Hence, it was not reduced by either short-term or long-term rapamycin treatment. These results suggest that the apical actin network is regulated by Rho-ROCK pathway independently of mTOR1 and mTORC2. Likewise, actin stress fibers in the basolateral side of cells were also decreased by ROCK inhibitor treatment in both of TSC1-deficit and -expression cells. Although the amount of active RhoA in whole cell was not increased in my assay, it is possible that spatial regulation of RhoA localization is regulated by TSC1. In addition, downregulation of Rac1 found in TSC1-expressing cells may also contribute to apical actin network. RhoA promotes apical constriction through the production of phospho-myosin regulatory light chain, whereas Rac1 suppresses this function of RhoA. Thus, my results suggest that the apical actin network in TSC1-expressing cells is induced by predominance of RhoA on Rac1 activity. I conclude that TSC1 may regulate the actin cytoskeleton via novel pathway, thereby play a role in polarity-related morphological regulation.


Tight junction (TJ)
apical
basal
TSC1 (-)
Tight junction (TJ)
apical
basal
TSC1 (+)


Tight junction
apical
basal
?
RhoA
TSC1
?
Rac1
TSC1
RhoA
TSC1
?